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Assuring the safe use of microbial biocontrol agents: a need for policy based on real rather than perceived risks

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Agriculture makes relatively little use of microorganisms which are introduced/applied for pest and disease control, yet such microorganisms represent enormous resources with proven potential as biological control agents. One reason for their underemployment is the lack of fundamental information on their biology and ecology, needed to achieve more consistent biological control. Another reason is the requirement that each strain, formulation, and use be registered based on a policy to treat microbes as "pesticides." The costs of registration, especially the costs of obtaining the necessary toxicological data patterned after requirements for chemical pesticides, rarely can be justified because the specific nature of microbial biocontrol agents limits their use to small and niche markets. Typically, a different biocontrol agent is needed for each pest or disease, or different agents are needed in different environments to control the same pest or disease. Studies are needed to identify the real risks of microbial biocontrol agents to human health and to the environment as a basis for appropriate regulation of these beneficial organisms. Furthermore, greater use should be made of microbial biocontrol, no matter how minor the use, including the use of genetically modified microbial biocontrol agents, so as to increase experience and familiarity with environmental uses of microorganisms among producers, processors, consumers, and the public.

Additional index words: sustainable agriculture, biotechnology, science policy.

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En agriculture, on utilise relativement peu les microorganismes pour la lutte biologique contre les maladies et les insectes, bien que ceux-ci représentent une immense ressource potentielle d'agents de lutte biologique. Le manque de connaissances fondamentales de leur biologie et de leur écologie, nécessaire pour la réussite d'une maîtrise constante, est l'une des raisons de leur sous-utilisation. Une autre raison est la politique qui fait qu'on traite les microbes comme des pesticides aux fins d'enregistrement des souches, des formulations et de leur utilisations. Les coûts d'enregistrement, particulièrement ceux nécessaires pour produire des données toxicologiques selon les mêmes exigenes que les pesticides chimiques, sont rarement justifiés car la nature spécifique des agents microbiens de lutte biologique restreint leur usage à des marchés à créneaux et petits. Pour chaque maladie ou insecte, il faut généralement un agent de lutte biologique distinct, qui peut même être différent selon le milieu. On a besoin d'études pour déterminer les risques réels des agents de lutte biologique sur la santé humaine et sur l'environnement pour développer des réglementations adaptées à ces organismes bénéfiques. De plus, il faudrait augmenter l'utilisation de la lutte biologique microbienne, à grande ou à petite échelle, y compris l'usage d'agents microbiens modifiés génétiquement, pour accoutumer et familiariser les producteurs, les transformateurs, les consommateurs et le public à l'usage environnemental des microorganismes.

Mots-clés additionnels: agriculture durable, biotechnologie, politique scientifique.

Microorganisms antagonistic to plant pathogens and naturally present in soil and on plants represent enormous but still largely untapped biological and genetic resources as agents to control plant diseases. The greatest use of these beneficial microorganisms in plant disease control, by far, is as communities of resident antagonists exploited as suppressive soils or through cultural practices such as crop rotations (8). The science and practice of plant pathology must continue to work towards greater understanding and towards ways to maximize the benefits of the communities of resident antagonists that provide biological control of plant diseases — the "background" level of natural biological control. However, full use of antagonists in biological control of plant pathogens also depends on the knowledge base and technology to introduce/apply individual or combinations of select strains when and where needed in the cropping system or plant disease cycle.

The means to deploy select strains of naturally-occurring or genetically modified microbial biocontrol agents against targeted plant diseases depends on advancements in at least five arenas (4). These are:

1. Science and education needed to develop and implement this technology;
2. Public and private investments in research and development;
3. Willingness of user groups, including producers, gardeners, foresters, and managers of urban and recreational areas and landscapes, to adopt this approach to improvement and protection of plant health;
4. The regulatory environment, including appropriate statutes, rules, and policy in place to assure safety to people and the environment;
5. Public perceptions of the safety of deliberate introduction/application of microorganisms onto/into plants, soils, and other environments.

Obviously, these five arenas are interdependent. Negative reactions to the use of microbial biocontrol from the general public or consumers results directly in more stringent regulations, including greater requirements for data on safety and longer periods for review prior to approval. More stringent regulations increase the cost of this technology, which directly impacts willingness of the user to adopt it. More stringent regulations also affect the willingness of the private sector to invest in this technology, whereupon the responsibility for research and development shifts onto public-sector investments. The key is science and education supported by public-sector investments in research and extension for the public good. Only through familiarity that comes from experience with and understanding of microbial biocontrol can we expect to allay public concerns, thereby giving some hope for relief in the regulatory arena and a cascade of other desirable outcomes on the road to greater use of microbial biocontrol.

Special challenges for microbial biocontrol

Over the years, plant pathology has indulged in a certain amount of self-examination on why there are so few examples of biological control of plant pathogens compared with biological control of insect pests. The answer rests mainly with the special challenges inherent with the use of microorganisms for biological control. Other than the use of resistant varieties, where plant pathology has been very successful, the biological agents available for use against the pathogenic fungi, bacteria, and viruses are other fungi, bacteria, and viruses. Biological control of plant parasitic nematodes with introduced agents likewise depends mainly on microorganisms. Other than *Bacillus thuringiensis*, which is used mostly as a natural-product pesticide, only a small percentage of the successful examples of biological control of insect pests are examples of microbial biocontrol. Whether the goal is to control insect pests, weeds, plant diseases, or plant parasitic nematodes, insect pathologists, plant pathologists, and nematologists face the same special challenges inherent with microbial biocontrol. Overcoming these challenges will open the way for one more major biologically based method for sustainable pest and disease control. Clearly, the promise of this emerging technology is worth the effort needed to make it work.

There are two major but interrelated challenges inherent with the use of microbial biocontrol. One is the technical difficulty of achieving consistent biological control with microorganisms, due in part to a lack of fundamental understanding of their ecology and biology, including the mechanisms of biological control. The other factor is that microorganisms have been or still are subject to regulations developed for chemical pesticides, a precedent set in the United States in 1948 with the registration of *Bacillus popilliae* for control of Japanese beetle under statutes of the U.S. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

The U.S. Environmental Protection Agency (EPA), which administers FIFRA, defines "biological control agent" as "any living organism applied to or introduced into the environment that is intended to function as a pesticide against another organism declared to be a pest by the Administrator" (40 CFR 152.3). The key word in this definition of biological control agent is "pesticide," which gives the EPA authority to regulate biological control agents under FIFRA. Under this authority, the EPA has exempted all biological control agents other than microorganisms from the requirements of FIFRA (40 CFR 152.20) on the basis that the other kinds of biological control agents, e.g. plants with resistance to pests, and arthropods and nematodes as natural enemies, are regulated sufficiently by other federal agencies.

Under FIFRA, each agent and each intended use of that agent is subject to a separate registration. There are more than 100 separate registrations of products based on *Bacillus thuringiensis* subsp. *kurstaki* dating back to when this microbial biocontrol agent was first registered under FIFRA in 1961 (9). This one agent alone accounts for more than half of all microbial biocontrol agents registered in the United States (under FIFRA) since 1948. Such repetitive registrations provide a means of record-keeping on the use of this agent, but would seem unnecessary if the intent is to protect human health and environment, which is the primary intent of FIFRA.

The challenge for microbial biocontrol becomes greater, based on current policy in most countries, if the agent has been genetically modified by recombinant DNA techniques. This situation continues to exist in spite of conclusions from several studies that the method of genetic modification does not determine the level of risk. One of the seminal reports on this issue, prepared for the U.S. National Academy of Sciences by the Committee on the Introduction of Genetically Engineered Organisms into the Environment, chaired by Arthur Kelman, makes a statement, supported by every study since this report, that "The risks associated with the introduction of R-DNA-engineered organisms are the same in kind as those associated with the introduction into the environment of unmodified organisms and organisms modified by other genetic techniques" (18).

Like any well-intentioned policy based on a need to satisfy one set of societal concerns, the impact over the long-term may include outcomes never intended. One effect is a shift in thinking about microbial biocontrol agents from a biological to a chemical paradigm (3). Even researchers have begun to think this way, referring to their agents as biopesticides, biofungicides, biofumigants, and mycoherbicides rather than biological control agents. Another outcome is the expectation that the candidate agent will work virtually 100% of the time and create a market large enough to justify the cost of registration. Consider, by comparison, that of an estimated 850 arthropods released as natural enemies of pests in the United States, about 40% have established and are providing some level of biological control (J.R. Coulson, personal communication). A 40% success rate is quite significant, but would not justify the cost of registration under a statute such as FIFRA.

Strains with ability to function over a wide geographic area or on several crops and to provide biological control virtually 100% of the time may be forthcoming in the future. In the meantime, many potentially useful strains remain in collections or have been lost because their limited effectiveness was taken to mean they did not work.

Microbial biocontrol, like resistant varieties, vaccines, beneficial insects, sterile-male insects, and other biologically-based methods of pest and disease control, is a generic technology where a different agent (or gene, vaccine, beneficial insect, or sterile male) must be identified, developed, and deployed for each pest or disease. The sterile-male technology developed to control screw worm in the southern United States and Mexico controls only screw worm. Likewise, the smallpox vaccine controls only smallpox, and the *pch* gene for resistance in wheat to *Pseudocercosporella* foot rot of wheat controls only this one disease of wheat (10). *Agrobacterium radiobacter* strain K84 controls only crown gall caused by *A. tumefaciens* and does not control the strain responsible for crown gall on grape vines (14,19). It is well worth noting that the specificity and limited utility of the genes and agents named above have not detracted from their recognized importance as scientific and technical achievements.

There are hundreds if not thousands of success stories of biologically-based approaches to safe pest and disease control. Microbial biocontrol holds this same promise. However, realizing this promise may not be possible if each strain, combination of strains, and intended use must be registered like a pesticide. Other more appropriate approaches must be developed to regulate microbial biocontrol agents. Realizing the promise of microbial biocontrol will also depend on the ability of researchers to develop improved strains (genotypes) of microbial biocontrol agents using the most appropriate methods.

Safety issues raised with the use of antagonists of plant pathogens

The primary purpose of this paper is to discuss safety considerations of antagonists intended for use as biological control agents of plant pathogens. The use of plant pathogens as biological control agents of weeds, and the use of insect pathogens as biological control agents of insects raise a different safety issue, namely the issue of pathogenicity to nontarget plants or insects. Antagonists as a group are saprophytes or they are parasitic on other microorganisms, e.g. mycoparasites. Antagonists of plant pathogens rarely if ever are pathogenic to plants, animals, or humans, although some may be weak or opportunistic pathogens. Those candidate antagonists with known potential as pathogens, e.g. clinically important strains of *Berkholdera cepacia* (26,27), can and should be eliminated during early phases of the screening program.

One approach to identification of safety issues raised by the use of antagonists introduced/applied to control plant pathogens is to examine the mechanism of antagonism. The mechanisms of antagonism of plant pathogens by microorganism are divided into three categories, namely, competition, antibiosis, and exploitation (1). Competition is mainly for one or more nutrients in limited supply, but can also be for space (2). Antibiosis is the inhibition of one microorganism by a substance produced by another microorganism (8). Exploitation includes the actual digestion/consumption of one microorganism by another, such as the parasitism of one fungus by another (mycoparasitism) and the predation of bacteria and fungi by protozoa and amoebae. None of these mechanisms of antagonism raise an issue of significant risk to plants, insects, or wildlife, especially when considered against the background of natural competition, antibiosis, and exploitation on-going wherever microorganisms co-exist, which is virtually every habitat on earth.

Appropriately, the requirements for registration of microbial biocontrol agents do not call for data on effects of microbial biocontrol agents on nontarget microorganisms. Obviously, researchers should be concerned that their antagonists introduced into the rhizosphere are compatible with nitrogen-fixing bacteria, in the case of legumes, and with mycorrhizal fungi, in the case of trees and crop plants that benefit from mycorrhizae. These are agronomic, horticultural, or silvicultural issues.

I am frequently asked about the nontarget effects of our antibiotic-producing rhizobacteria (24) introduced into the rhizosphere of wheat for control of take-all (5) on nontarget microorganisms in the rhizosphere of wheat. I begin my answer with another question: Compared to what? Every option available to control this disease, including crop rotation, intensive tillage, incorporating chicken manure, such as recommended earlier this century in Kansas by Fellows and Ficke (12), or waiting for take-all decline (21), all unavoidably and naturally affect which microorganisms are likely to establish in the rhizosphere of wheat. Even allowing take-all to develop unchecked will influence which microorganisms are likely to establish in the rhizosphere of wheat.

It would seem likely that the antibiotic-producing rhizobacteria introduced for biological control of take-all will establish in place of rather than in addition to other rhizobacteria, possibly in place of other fluorescent pseudomonads less able to protect wheat against take-all. Kloepper and Schroth (16) showed that the populations of several groups of rhizosphere microorganisms were reduced in response to the introduction of certain strains of rhizobacteria. This kind of displacement/preemption is typical of the dynamics expected of populations and communities of microorganisms in response to natural and unnatural perturbations.

Rather than possible effects on the environment, the issues raised by releasing antagonists intended for use in biological control relate mainly to human health. Firstly, there is the issue of possible effects on workers in production facilities and those who apply these agents. Secondly, there is the issue of safety to people who eat fruits and vegetables treated with antagonists to prevent storage rots (25), such as the antagonists *Candida oleophila* registered as Aspire® and two strains of *Pseudomonas syringae* registered, respectively, as Bio-Save 10® and Bio-Save 11® (9).

In many if not most cases, the risks of the antagonist to human health, if any, can be predicted from the scientific literature based on knowledge of the species or taxonomic group that contains the species, e.g. spores of fungal antagonists likely to cause allergies in people or clincally important strains of *B. cepacia*. If risk to workers and consumers is a significant safety issue, then research is needed specifically to address this issue. This kind of research would seem more useful to address the question of safety rather than studies on nontarget effects of antagonists in specific habitats, such as the rhizosphere, that are neither of environmental consequence nor ecologically unique.

Safety issues raised with the use of genetically-engineered antagonists of plant pathogens

The public now widely accepts the safety of products developed in medicine using the new tools of biotechnology. Even with the products of biotechnology developed for use in agriculture, the debate is shifting from safety concerns to social concerns, including concerns for animal rights, survival of small farms, and whether this technology will accelerate the trend towards so-called "industrialized agriculture." However, the idea of releasing genetically engineered microorganisms into the environment continues to conjure images of microbes that cannot be controlled. These fears were fueled in the 1980s in the cases of the field-releases of ice-minus bacteria on potatoes and strawberries in California when the EPA required the field workers to wear moon suits complete with face masks when working physically in the plots — hardly reassuring to an already skeptical public. These kinds of requirements have been mostly discontinued, but the EPA maintains authority to regulate/approve any field releases involving strains of microorganisms genetically modified by rDNA techniques to enhance biological control. The EPA defers to the USDA's authority under the U.S. Federal Plant Pest Act to regulate/approve field releases on naturally occurring microbial biocontrol agents that involve less than 10 acres of land or 1 acre of water.

It is worth reminding ourselves that the crops and livestock used in agriculture today trace back to wild species of plants and animals, and that the varieties and breeds in use are the result of selection, domestication, and in most cases decades of breeding. With virtually every kind of crop and livestock used in agriculture today, some traits have been enhanced and others eliminated to suit the needs of producers, processors, and/or consumers. Throughout this effort, two outcomes are clear: a) the food and other products produced from these plants and animals have become safer for people and b) the plants and animals subjected to this breeding and artificial selection have become more dependent on human nurturing to survive.

Likewise with microbial biocontrol agents, we can expect that selection, "domestication," and genetic modification of strains to fit specific needs of producers, processors, and consumers will produce agents both safer to people and more dependent on human nurturing to survive. Strain K1026 of *A. radiobacter*, developed in Australia for biological control of crown gall (20), is the first genetically engineered microbial biocontrol agent in commercial use in the world. This strain lacks the ability to transfer its resistance to the antibiotic agrocin 84 to the target pathogen, thereby further reducing the chances that a population of the pathogen could emerge with resistance to this microbial biocontrol agent. With each kind of microbial biocontrol agent, desirable traits can be added or enhanced and undesirable traits can be attenuated or eliminated; r-DNA techniques allow researchers to make these genetic changes very precisely.

Regulatory agencies and some environmental activist groups have called for special or additional regulations to be applied to genetically engineered compared with naturally occurring microorganisms on the basis that microorganisms have the potential to spread, multiply, and maintain their populations in the environment. Genetically engineered microorganisms are therefore considered like nonindigenous microorganisms as exotic and hence intrinsically of unknown risk. Even strains of fluorescent pseudomonads with the *lacZY* marker system (11,17) were initially subjected to regulations that did not apply to the wild-type parents, but recent policy proposed by the U.S. EPA would focus future regulations on strains with enhanced activity as microbial biocontrol agents.

If worker safety is a concern, as indicated above, then microbial biocontrol agents genetically modified to enhance ability to establish and maintain an effective population within a field, orchard, forest, or other environment where needed, could reduce the need for workers to produce and introduce that agent. Such modification would lessen the risk to workers. In this regard, the U.S. EPA has deferred to the USDA in the case of some insect pathogens imported from the geographic "home" of the target insect pest and introduced into the United States with the intent that it establish, spread, and maintain its population on the target insect population (classical biological control). In essence, this policy recognizes that the current limit of 10 acres of land and 1 acre of water for introductions/applications without EPA approval is moot with microorganisms that establish parasitically and spread epidemiologically/epizoologically on a population of targeted weeds or insect pests.

Actually creating an antagonist with increased ability to establish and spread could be technically very difficult, considering the genetic complexity and our poor understanding of the traits and characteristics that confer ability to compete and survive in the environment. Consider further that the overriding lesson with antagonists intended for use in biological control of plant pathogens is that alien species and strains establish in foreign environments/habitats only with great difficulty if at all, and maintenance of timely effective populations usually depends upon regular augmentative applications.

Considering the need for participation of the private sector in implementation of microbial biocontrol, and that businesses depend on repeat sales, and also considering the likely near-term public concerns for microorganisms that are genetically modified to survive longer and spread in the environment, it would seem prudent that our research focuses on other kinds of improvements in the performance of antagonists for use as biological control agents of plant pathogens. One such improvement with plant-associated microorganisms would be to enhance their ability to establish and maintain high populations on the plants targeted for protection. Such an improvement should be designed so as to confer no advantage to the strain when the targeted plants are not grown, thereby assuring that the strain could not survive in the absence of its host(s).

Enhanced antibiosis would be another kind of improvement, whether against a target pathogen or as a broader-spectrum of activity so as to control a complex of pathogens (24). Kim et al. (15) have shown that *P. fluorescens* Qc-69, with biocontrol activity against wheat take-all, exhibited greater biocontrol activity under controlled conditions when transformed with genes either for biosynthesis of phenazine-1-carboxylate or 2,4-diacetylphloroglucinol. These two antibiotics account for most of the biocontrol activity of strains 2-79 (22) and Q2-87 (23), respectively, whereas the wild-type Qc-69 does not produce either of these antibiotics (D. M. Weller, unpublished). Interestingly, the transformed strains provided a given level of biological control at a lower inoculum load on the seed, compared with the parent strain. This has implications for both the economics of this technology and worker safety, since less material must be produced and handled to produce a given effect. On the other hand, but not surprising, transformed strains that produced the most antibiotic tended to survive the poorest. We can expect that the enhancement or addition of a trait such as ability to produce an antibiotic will carry a price to the transformed microorganism. Knowledge of the genetics of saprophytic fitness, including ability to use diverse substrates and multiply over a range of environmental conditions in competition with indigenous microorganisms may be critical just to maintain wild-type levels of these characteristics in antagonists with enhanced ability to provide biological control through antibiosis.

Gaining familiarity through greater use of microbial biocontrol

A major factor in public acceptance of products developed with the new tools of biotechnology in medicine is the increase in experience and hence familiarity with these products and their use. Most importantly, these products offer the potential for direct benefits to our personal health, or the health of our families, and therefore we know more about them and are more willing to try them. The public also has considerable familiarity with the use of microorganisms to produce foods and beverages, e.g. cheeses, yogurts, sausages, wine, and beer, and their use to produce antibiotics, including genetically engineered strains for some of these applications (6). Again, the personal benefits are direct in the form of food, drink and medicines. However, familiarity is lacking for microbial biocontrol, especially for microbial biocontrol with agents developed with the new tools of biotechnology. Moreover, while the public expects agriculture to reduce its dependency on chemical pesticides, public perceptions are that microbial biocontrol could represent a different if not worse kind of risk, and further that the benefits of products developed with the tools of biotechnology will be mainly to stockholders of biotechnology companies and not to consumers.

The best solution to this dilemma is to make greater use of and gain more public attention for microbial biocontrol, no matter how minor the application. Biological control of crown gall with strain K84 is a model: it works, it offers a limited and usually local business opportunity in the country or region where used, it is safe to both workers and the environment, and it gives new meaning to the term "minor use." Kaiser et al. (13) have identified several strains of *P. fluorescens* with potential to protect seeds of chickpea against pythium damping-off during germination in soils in the Inland Pacific Northwest. This region of the United States only grows about 4000 hectares of chickpeas, and such a small industry normally would not justify the economics of developing and registering a microbial biocontrol product. Yet the agent identified by Kaiser et al. (13) works, it would offer a local business opportunity to supply the product, it is safe to workers and the environment, and it would be compatible with the use of rhizobium inoculations of seed for nitrogen fixation.

Countless examples of candidate microbial biocontrol products exist throughout the network of public supported research universities and federal laboratories of the developed and many developing countries. It is only through implementation, including entirely by public-sector efforts for the public good, if necessary to overcome initial barriers or develop the necessary infrastructure, can we expect the use of microbial biocontrol to grow. Only with growth in this area of science, and with greater experience in the use of microbial biocontrol, can we expect to gain greater familiarity with this technology among user groups, private investors, regulatory agencies, and, most importantly, the public.

In 1993, in a presentation at the Plenary Session of the 6th International Congress of Plant Pathology in Montreal, I issued a challenge to plant pathologists (7) that I will repeat here. My challenge is that plant pathologists provide the scientific leadership in the use of beneficial microorganisms in agriculture. This challenge does not call for a change of direction for our discipline, only a broadening of what plant pathology is already — the discipline of understanding and managing specific species, varieties, strains, and genotypes of microorganisms in the environment. Plant pathology is already the source of principles, concepts, and theory on the ecology and practical management of undesirable microorganisms—plant pathogens—in the environment. These same principles, concepts, and theory apply to understanding the ecology and managing specific genotypes as well as communities of beneficial microorganisms that have so much to offer the future of food and agriculture.

1. **Baker, R.** 1968. Mechanisms of biological control of soil-borne pathogens. Annu. Rev. Phytopathol. 6:263–294.
2. **Clark, F.E.** 1954. The concept of competition in microbial ecology. Pages 339–347 *in* K. F. Baker and W. C. Snyder, eds., Ecology of Soil-borne Plant Pathogens. Univ. Calif. Press, Berkeley, CA. 571 pp.
3. **Cook, R.J.** 1991. Success with biological control requires new thinking by industry. IMPACT Ag Bio Business. June 1991, pages 3–4. CAB International, Wallingford, Oxon, UK.
4. **Cook, R.J.** 1993. The role of biological control in pest management in the 21st century. Pages 10–20 *in* R. D. Lumsden and J. L. Vaughn, eds., Pest Management: Biologically Based Technologies: Proceedings of the Beltsville Symposium XVIII, Am. Chem. Soc. Washington, DC. 435 pp.
5. **Cook, R.J.** 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. Annu. Rev. Phytopathol. 31:53–80.
6. **Cook, R.J.** 1994. Biotechnology applied to microbes: taking agricultural sustainability a step forward. The World & I, a publication of The Washington Times Corporation. 9(12):369–383.
7. **Cook, R.J.** 1996. Sustainable agriculture: introduction and summary. Can. J. Plant Pathol. 18:115–118.
8. **Cook, R.J.**, and **K.F. Baker.** 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN. 539 pp.
9. **Cook, R.J., W.L. Bruckart, J.R. Coulson, M.S. Goettel, R.A. Humber, R.D. Lumsden, J.D. Maddox, M.L. McManus, L. Moore, S.F. Meyer, P.C. Quimby, Jr., J.P. Stack,** and **J.L. Vaughn.** 1996. Safety of microorganisms intended for pest and plant disease control: a framework for scientific evaluation. Biol. Control 7: 333–351.
10. **Doussinault, B., A. Delibes, R. Sanches-Monge,** and **F. Garcia-Olmedo.** 1983. Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. Nature 303:698–700.
11. **Drahos, D.J., B.C. Hemming,** and **S. McPherson.** 1986. Tracking recombinant organisms in the environment: β-galactosidase as a selectable non-antibiotic marker for fluorescent pseudomonads. Bio/Technology 4:439–444.
12. **Fellows, H.,** and **C.H. Ficke.** 1934. Wheat take-all. Kansas Agric. Exp. Sta. Annu. Rep. 1932-34:95–96.
13. **Kaiser, W.J., R.M. Hannan,** and **D.M. Weller.** 1989. Biological control of seed rot and preemergence damping-off of chickpea with fluorescent pseudomonads. Soil Biol. Biochem. 21:269–273.
14. **Kerr, A.,** and **C.B. Panagopoulous.** 1977. Biotypes of *Agrobacterium tumefaciens* and their biological control. Phytopathol. Z. 80:172–179.
15. **Kim, D.-S., R.F. Bonsall, L.S. Thomashow,** and **D.M. Weller.** 1995. Construction of transgenic *Pseudomonas fluorescens* Q69c-80 for improved biocontrol activity of take-all. Phytopathology 85:1146.
16. **Kloepper, J.W.,** and **M.N. Schroth.** 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology 71:1010–1024.

17. **Kluepfel, D.A., D.L. Kline, H.D. Skipper, T.A. Hughes, D.T. Gooden, D.J. Drahos, G.F. Barry, B.C. Hemming,** and **E.J. Brandt.** 1991. Release and tracking of genetically engineered bacteria in the environment. Phytopathology 81:348–352.
18. **National Research Council.** 1987. Introduction of recombinant DNA-engineered organisms into the environment: Key issues. National Academy Press, Washington, D.C. 24 pp.
19. **Perry, K.L.,** and **C.I. Kado.** 1982. Characteristics of Ti plasmids from broad host range and ecologically specific biotype 2 and 3 strains of *Agrobacterium tumefaciens*. J. Bacteriol. 151:343–350.
20. **Ryder, M.H.,** and **D.A. Jones.** 1990. Biological control of crown gall. Pages 45–63 *in* D. Hornby, ed., Biological Control of Soil-borne Plant Pathogens. CAB International, Wallingford, Oxon, U.K. 479 pp.
21. **Shipton, P.J.** 1975. Take-all decline during cereal monoculture. Pages 137–144 *in* G. W. Bruehl, ed., Biology and Control of Soil-borne Plant Pathogens. Am. Phytopathol. Soc., St. Paul, MN. 216 pp.
22. **Thomashow, L.S.,** and **D.M. Weller.** 1988. Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bacteriol. 170:3499–3508.
23. **Vincent, M.N., L.A. Harrison, J.M. Brackin, P.A. Kovacevich, P. Mukerji, D.M. Weller,** and **E.A. Pierson.** 1991. Genetic analysis of the antifungal activity of a soilborne *Pseudomonas aureofaciens* strain. Appl. Environ. Microbiol. 57:2928–2934.
24. **Weller, D.M.,** and **L.S. Thomashow.** 1993. Microbial metabolites with biological activity against plant pathogens. Pages 173–180 *in* R. D. Lumsden and J. T. Vaughn, eds., Pest Management: Biologically Based Technologies, Proceedings of Beltsville Symposium XVIII. Am. Chem. Soc., Washington, D.C. 435 pp.
25. **Wilson, C.L.,** and **M. Wisniewski.** 1989. Biological control of postharvest diseases of fruits and vegetables: an emerging technology. Annu. Rev. Phytopathol. 27:425–441.
26. **Yohalem, D.S.,** and **J.W. Lorbeer.** 1994. Multilocus isoenzyme diversity among strains of *Pseudomonas cepacia* isolated from decayed onions, soils, and clinical sources. Syst. Appl. Microbiol. 17:116–124.
27. **Yohalem, D.S.,** and **J.W. Lorbeer.** 1994. Intraspecific metabolic diversity among strains of *Burkholderia cepacia* isolated from decayed onions, soils, and the clinical environment. Anton. Leeuwenhoek Int. J. Gen. M. 65:111–131.